# Beyond communication

## VALORISATION

*"The importance of asking questions purely out of curiosity and carrying out experiments to test models that arise from these questions—the paradigm of basic biological research—cannot be dismissed as 'old-style' thinking; it is the main driving force of scientific freedom and originality."* - Schvartzman [1] -

# 1. INTRODUCTION

This chapter will provide an overview on the valorisation of this thesis. This was done in accordance with the guidelines introduced by the Maastricht University in 2014 that state all dissertations should include a chapter on the valorisation of the research. Valorisation of research lays in the potential use of its outcomes, so if these outcomes are of direct or indirect benefit for society. Or as defined by the national valorisation committee: "valorisation is the process of creating value from knowledge by making knowledge suitable and/or available for economic and/or societal use and translating that knowledge into competitive products, services, processes, and entrepreneurial activity" [2].

It is easy to realize a valorisation of research biased by valorisation policies with a defined measureable outcome set a priori. This type of research usually concerns well designed research, marked-out as a controlled trajectory. If you would compare designing and realising a research project with the design and realization of a building design this would almost guarantee you to achieve the plotted end product. However, sometimes one needs to make a leap beyond the practical and set out on an exploration. This, in part, applies for basic research that aims at identifying and describing biologic processes to deepen understanding. In my opinion, the gain of fundamental knowledge on a subject, in itself should be its valorisation. Because isn't it so that an increasing knowledge of a phenomenon usually leads to the valorisation of the phenomenon.

This study focused on small membrane 'bags' once thought of as garbage bags observed to be released by certain cells of our body. Also in the field of microbiology the release of the membrane vesicles was reported. To date, around 30 years after their discovery, the value of these membrane vesicles is being increasingly recognized. It is now clear that the release of these vesicles is a highly-conserved process that occurs by cells that belong to all branches of life. They are involved in intercellular communication and processes that lay beyond, such as the acquisition of nutrients and the protection against environmental factors. Hereby their physiological activities affect and enable a wide range of biological processes. In this thesis, we investigated the release of membrane vesicles in an *in vitro* infection-model based on macrophages and bacteria associated with airway disease to obtain a better understanding of the biological significance of vesicles shed by the host and bacteria during infection.

## 2. RELEVANCE FOR SOCIETY

Respiratory infections have a large impact on the global health. The World Health Organisation established that acute respiratory tract infections are the most important cause of morbidity and mortality worldwide and account annually for 4 million deaths [3]. Besides the direct consequences for patients that succumb to such an infectious disease, there is also a substantial economic burden. Over the years, a better understanding of the determinants for the process of bacterial invasion, manifestation, and spread has led to improved means to fight infection. As was reviewed in this thesis (chapter 2) and by others [4,5], bacteria release membrane vesicles that provide them with a means to cope with host cell-associated environmental challenges, both during bacterial growth and during infection. Membrane vesicles contain e.g. lipopoly- or lipooligosaccharides and a multitude of proteins and they can aid in the acquisition of nutrients and the defence against antimicrobial peptides. Importantly, host-associated environmental stressors (such as nutrient limitation and the exposure to antimicrobial peptides) can trigger the release of membrane vesicles. In this thesis we studied the release and functionality of membrane vesicles released during macrophage infection with respiratory pathogens. Additionally, we determined the effects of frequently prescribed pharmacological agents and a promising new regimen on the vesicle shedding. We and others observed that the application of pharmacological regimens may substantially boost the vesicle production (chapters 5 and 6, [6,7]), thereby liberating a multitude of vesicle-associated virulence factors into the host-environment [8,9]. Future studies that focus on bacterial behaviour and growth in response to therapeutics and host factors can help to predict (or explain) treatment outcome and may aid in the development of novel therapeutics.

# 3. VALORISATION OF PROJECT OUTCOMES

## 3.1 Communication

As mentioned previously, the research field of membrane vesicles is relatively young and dynamic. The value of these vesicles becomes increasingly recognized and it is now understood that they are involved in a plethora of pathologies including infectious diseases. A critical appraisal on the release and physiological activity of bacterial membrane vesicles in the context of a host-environment is given in chapter 2, which has been published in Critical Reviews in Microbiology. The studies presented in this thesis provide novel insights in the release of bacterial and host cell membrane vesicles during infection and the findings presented in chapters 2-6 of this thesis have been made available for the scientific society through publications in peer-reviewed journals.

Moreover, most of the results obtained during these studies were communicated at several international conferences. Especially the sharing of experiences, results, and techniques during the annual conference of the International Society for Extracellular Vesicles was highly valuable. As this field is still rather young and involves many research fields it is particularly constructive to exchange information with fellow researchers that work within the same niche. Finally, as this project was a pioneering project at the department of Medical Microbiology, the experimental techniques and results this project delivered facilitate follow-up projects and opened new possibilities for collaboration between the departments' research groups.

## 3.2 Activities, products, and innovation

Researchers that work with membrane vesicles face the challenge of working in a field that is subject to constant change and expansion, foremost with respect to the techniques used to study these vesicles. Oftentimes, highly specialized techniques are required for the study of membrane vesicles and technical limitations can hamper assessment of their release and functionality, techniques and experimental parameters are constantly evolving. As a consequence, there were several shifts in consensus as to which techniques were best suited for the isolation and purification as well as for the characterization. To reach consensus on this an ISEV position paper was published several years ago followed by the establishment of the EV-TRACK consortium, this year. This consortium established a knowledgebase that facilitates the registration of a study and its research procedures, hereby not only improving clarity and easing the interpretation of studies, but also providing with guidelines to improve reporting. The studies presented in this thesis were all registered in this database.

Often, existing techniques to study membrane vesicles need to be customized to enable addressing specific research questions. Also in this study, we had to overcome a technical limitation by customizing a conventional method in order to be able to assess the release of specific membrane vesicle populations within a mixed vesicle population. To this end, we developed a semi-quantitative method that allowed us to determine the release of bacterial vesicles. The manuscript in which this application was presented (chapter 3) was published in Microbiology Research. The introduction of this method provides a new way to study bacterial vesicle release during infection and allows e.g. to establish

the impact how host environment-associated factors affect bacterial membrane vesicle release. Moreover, this method can be taken over directly by researchers in the field as it provides with a platform that can easily be adopted for alternative applications and is therefore highly accessible and versatile.

Finally, innovation is being described as the application of an invention. Inventing literally means to create a new concept or conception. One of the concepts or take-home messages of this thesis is that a host-environment can alter bacterial behaviour. In this thesis, we focussed on one aspect of this behaviour, namely the release of membrane vesicles. In chapter 2 we introduced the concept that the transformation of the bacterial outer membrane and the release of membrane vesicles can provide bacteria with a selective advantage in the host environment. In chapter 3, we studied this by assessing the bacterial membrane vesicle release in the context of infection. We took a first step to study how the interaction with host cells may affect the bacterial membrane vesicle release in chapters 4-6. The discussion of this thesis provides with directions for future research. We think studying this concept will eventually help us to understand how bacteria succeed in overcoming host defence mechanisms and thrive in a host environment, because it is in the interaction with the host that their pathogenicity is determined.

## REFERENCES


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